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Role of assays of total estriol in amniotic fluid for the diagnosis of fetal hypotrophy

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1 Introduction

In hypotrophic fetuses and newborn infants perinatal morbidity and mortality are much increased compared to those in eutrophic infants [11, 13]. In severe cases, brain development in the small-for-date babies may be impaired [4, 5, 14, 22, 23].

Detection of these high-risk infants and assessment of their well-being prior to birth are of considerable interest to the obstetrician since he may hereby obtain useful indications for therapy.

The diagnosis of fetal hypotrophy is now generally made by ultrasonic measurement of fetal size and hormone assays. In this connection, estriol is one of the compounds in amniotic fluid which has attracted attention. Estriol, which is synthesized in very large amounts during late pregnancy, is almost entirely a product of the feto-placental unit [3]. Since amniotic fluid is free of direct maternal influences, it is conceivable that its estriol concentrations will reflect current fetal and placental function and fetal development.

The major objective of this study has been to examine estriol concentrations in amniotic fluid in eutrophic and hypotrophic infants and then to clarify whether and to what extent estriol assays in the liquor may contribute to the antenatal identification of intrauterine hypotrophy.

2 Patients, materials and methods

In 108 pregnancies (gestational weeks 31 to 41) 127 samples of amniotic fluid were assayed for their concentration of total estriol. Antepartally the samples were obtained by transabdominal amniocenteses, indicated for various clinical reasons; subpartally amniotic fluid was withdrawn following transcervical puncture of the amniotic cavity. Among the pregnancies examined there were 74 pregnancies with eutrophic infants (Group A) and 34 with hypotrophic infants (Group B). The infants were classified in one of the two groups by gestational age and birthweight [12]. Gestational age was determined by means of menstrual history, the first maternal perception of fetal movements and the degree of maturity immediately after delivery, the estimation of which was made according to the clinical criteria proposed by FARR and PETRUSSA [6, 17]. For the assessment of the birthweights of the neonates at a particular gestational age the birthweight curves according to NICKL were employed [16]; they describe the relative frequency of neonatal birthweights by week of gestation for the present German population. Hypotrophic neonates were defined as infants whose birthweights lay below the 3rd percentile according to NICKL.

Total estriol in amniotic fluid was assayed by a chemical method for highly specific estriol determination [1]. The special features of the assay are as follows:

a) clear-cut TLC-separation of estriol from the rest of the phenolic fraction of the sample,
b) characterization of the sample estriol spots by comparison of position with reference estriol spots run in parallel,

c) direct quantification of estriol spots by reflectance spectrophotometry on the chromatogram,
d) computer-assisted evaluation of the recorded reflectance values applying the KUBELKA-MUNK equation.

The high specificity of the estriol assay used results from the direct measurement within the chromatographically pure estriol spot. In a range from 40 to 400 ng estriol per spot, the precision of the method was ± 4 per cent. The recovery rate of known amounts of estriol added to sample material prior to assay varied from 70 to 80 per cent. With amounts of 40 ng estriol per spot, the lower limit of sensitivity for reliable reflectance measurements was reached. For total estriol assays by means of the above-cited method, amniotic fluid samples of 5 to 10 ml were used. Until processing, the samples were stored at – 20°C.

3 Results and comments

A survey of the clinical data concerning the groups examined is given in Tabs. I and II. The mean weight of the hypotrophic neonates was 758 g lower than that of the eutrophic neonates, whereas the mean placental weight in the hypotrophic group was by 138 g lower than that in the eutrophic group. The clinical status of the hypotrophic infants immediately post partum, as judged by our modified score [18], was diminished in comparison to that of the eutrophics; in addition, the hypotrophic neonates had more frequently been developing acidoses. One of the 74 eutrophic infants died within 24 hours after delivery (1.4 per cent), on the contrary, 3 of 34 hypotrophic infants perished during the perinatal period (8.8 per cent).

The analysis of the maternal, pregnancy-associated disturbances and diseases is rather interesting. The complication rate in the mothers of the eutrophic neonates was 5.4 per cent (2 cases of gestational diabetes mellitus, 1 case of abortus imminens, mens IV, 1 case of active toxoplasmosis; n = 74). In contrast, the complication rate in the mothers of the hypotrophic infants amounted to 55.9 per cent, i.e. approximately tentimes the former complication rate (14 cases of toxemia, 4 cases of gestational diabetes mellitus, 1 case of abortus imminens, mens III, 1 case of vitium cordis; n = 34).

Figs. 1 and 2 show the distribution of the concentrations of total estriol by age of gestation in the two groups examined. **With advancing gestational age a conspicuous rise of total estriol concentrations in amniotic fluid** is to be encountered. At gestation weeks 37 to 41, at "term", the mean total estriol values are more than twice as high as those in the four gestational weeks before (Tab. III). This pattern is valid for the pregnancies with eutrophic infants as well as for those with hypotrophic progeny.

In general the total estriol concentrations in amniotic fluid in pregnancies with hypotrophic infants lie much lower than those in pregnancies with eutrophic infants. The differences are statistically significant ($P < 0.001$, $\chi^2 = 28.55$ and 22.33 respectively, 1 degree of freedom). **The mean values of total estriol in amniotic fluid in the hypotrophic group amount to only 42 and 43 per cent of those in the eutrophic.**

There is an overlapping in the estriol ranges of both groups. This implies that a normal total estriol concentration measured in amniotic fluid does not necessarily exclude fetal hypotrophy.

Tab. I. Clinical data on the groups investigated.

	Eutrophic neonates	Hypotrophic neonates
Mean body weight (g)	3149	2391
Mean placental weight (g)	566	428
Saling score (points, $\bar{x}$)	9.8	8.5
Vaginal delivery	85 %	71 %
Sectio caesarea	15 %	29 %
Total number of infants examined	74 (=100%)	34 (=100%)

Tab. II. Disturbances immediately post partum in the neonates examined. – Depression ≤ 6 points of the score according to SALING [18]. Respiratory acidosis = pH act. ≤ 7.19 and CO_2 overload in arterial cord blood. Metabolic acidosis = pH qu40 ≤ 7.19 in arterial cord blood.

	Eutrophic neonates	Hypotrophic neonates
Depression of the neonate immediately post partum	3 = 5.4%	11 = 32.4%
Respiratory acidosis immediately post partum	6 = 8.1%	6 = 17.7%
Metabolic acidosis immediately post partum	3 = 4.0%	4 = 11.8%
Total number of infants examined	74 = 100%	34 = 100%

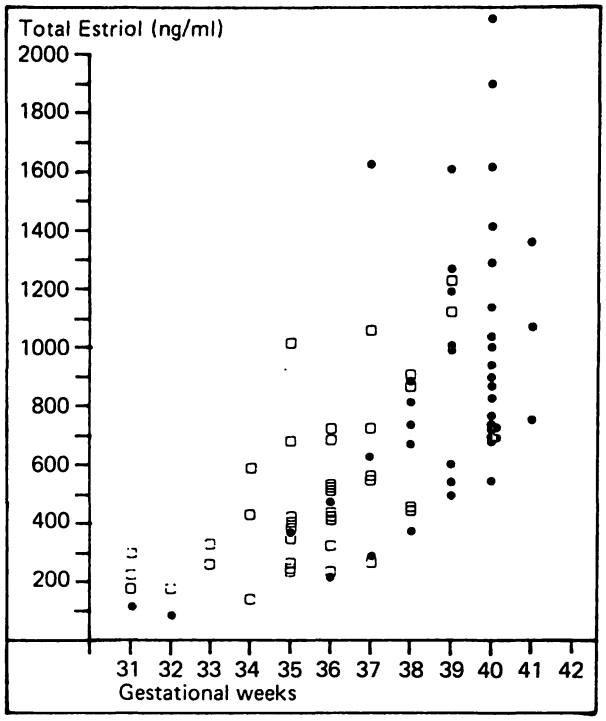

Fig. 1. Distribution of total estriol concentrations in amniotic fluid, related to gestational age: Pregnancies with eutrophic infants. Squares = antepartal values; dots = subpartal values.

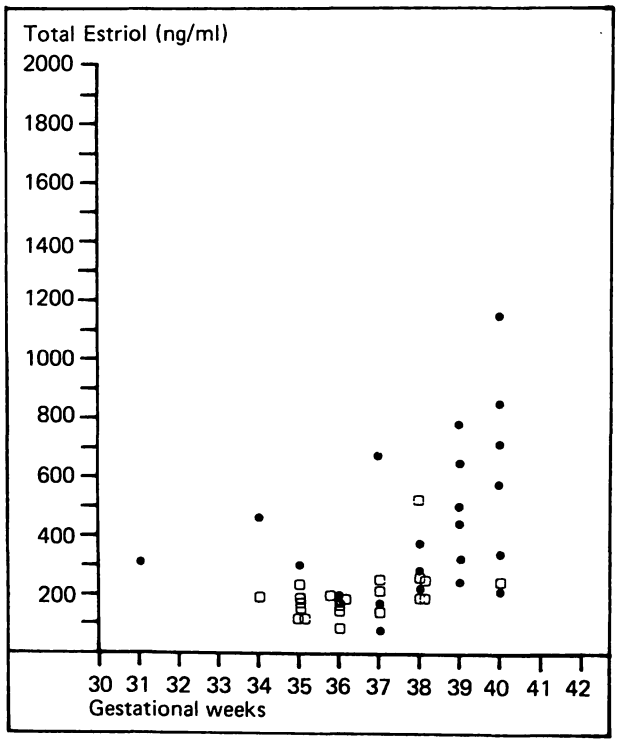

Fig. 2. Distribution of total estriol concentrations in amniotic fluid, related to gestational age: Pregnancies with hypotrophic infants. Squares = antepartal values; dots = subpartal values.

Nevertheless, some clinically remarkable features of the present data material must be emphasized. At gestational weeks 33 to 36 only one out of 29 estriol values in the eutrophic group lies below the level of 200 ng per milliliter, at weeks 37 to 41 three out of 48 estriol values lie below the level of 400 ng per milliliter of amniotic fluid. On the other hand, at gestational weeks 33 to 36 not a single one out of 16 estriol values in the hypotrophic group exceeds the level of 500 ng per milliliter, at weeks 37 to 41 one out of 26 values in the same group lies above the threshold of 1000 ng per milliliter of amniotic fluid.

The mean total estriol values in amniotic fluid obtained from term babies at the day of delivery (Tab. IV) are slightly greater than the corresponding values in Tab. III which represent estriol values obtained antepartally and subpartally. These findings support the view that, near term, total estriol concentrations continue to rise up to the day of delivery, as they also do in normal pregnancies and in pregnancies with hypotrophic infants.

Comparison of the mean birthweights in the eutrophic and hypotrophic group and comparison of the mean total estriol concentrations in amniotic fluid in both groups show noteworthy results. While the mean birthweight in the hypotrophic term neonates amounts to 73 per cent of that in the eutrophic term neonates, the mean estriol concentration in the hypotrophic group amounts to only 47 per cent of the corresponding value in the eutrophic group. In other words, compared to the eutrophic babies, the growth deficit in the hypotrophic babies (27 per cent) is much smaller than their estriol deficit (53 per cent) in amniotic fluid. This finding supports our assumption that estriol assays in amniotic fluid are an useful tool in diagnosing intrauterine hypotrophy.

4 Discussion

Determinations of estriol in maternal urine and plasma are generally accepted by obstetricians as being useful ways of following the condition of the fetus. Examination of amniotic fluid is the closest diagnostic approach which can be made to the fetus in situ. As is known, the steroid hormone patterns in the fetus and in amniotic fluid are very

	Eutrophic infants (A)		Hypotrophic infants (B)			
Gest. weeks	n	Total estriol (ng/ml) $\bar{x} \pm s$	n	Total estriol (ng/ml) $\bar{x} \pm s$	Statistical significance	$\frac{E\ 3\ \bar{x}\ (B)}{E\ 3\ \bar{x}\ (A)}$
37–41	48	943.8 ± 464.8	27	392.2 ± 258.9	$p<0.001$	0.42
33–36	29	430.0 ± 191.7	16	185.7 ± 86.2	$p<0.001$	0.43
30–32	6	176.7 ± 78.2	1	*)	—	—

Tab. III. Comparison of total estriol concentrations in amniotic fluid in pregnancies with eutrophic and hypotrophic infants. Calculations of statistical significance of the estriol differences by means of X^2-test; n = number of amniotic fluid samples assayed. x) 1 value only (see Fig. 2).

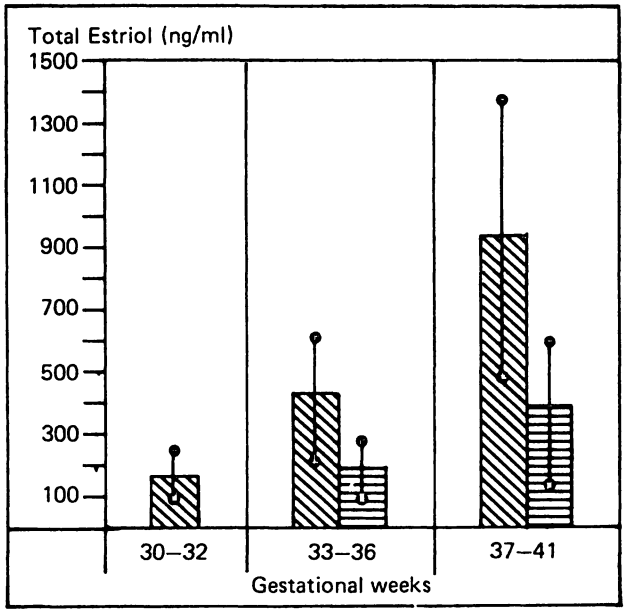

Fig. 3. Concentrations of total estriol in amniotic fluid; arithmetical means and standard deviations.
Values in eutrophic infants = diagonally hatched columns; values in hypotrophic infants = transversely hatched columns.

similar to each other. The steroids present in amniotic fluid chiefly originate from the excretions of the fetus – mainly from its micturition – and there exists a dynamic equilibrium between amniotic fluid and maternal circulation [2, 19, 20]. Thus, it may be suggested that estriol concentrations in amniotic fluid reflect the current fetal and placental function to a certain degree. There are also good reasons for supposing that maternal influences such as her metabolism and excretory functions, intake by the mother of fluids, alcohol, nicotine, drugs etc. produce more attenuated effects upon the estriol conditions in the fetoplacental unit than upon those in maternal plasma and urine.

The total estriol concentrations in the amniotic fluid which we measured in eutrophic infants are in agreement with representative estriol data from other authors [7, 9, 19].

In both groups investigated the rise in the estriol concentrations is persistent up to the day of delivery. The scatters of the estriol values are large. According to the result of this investigation it is beyond question that as a rule hypotrophic infants have significantly lower estriol concentrations in amniotic fluid than eutrophic infants at the same gestational age.

Considerable diagnostic uncertainities result from the fact that there is a good deal of overlap between the estriol concentrations in amniotic fluid of eutrophic and those of hypotrophic infants. Indeed, in cases showing low-normal estriol values in amniotic fluid one cannot safely distinguish by the estriol values alone whether the appropriate fetus is eutrophic or not. In such a situation a diagnostic assessment of the estriol data can be tried by combining them with other chemical and clinical findings in the pregnancy under examination. For the correct assessment of the estriol values it is important that the antenatal calculation of gestational age is accurate.

As already mentioned, definite marginal estriol values are of great diagnostic value. **At gestational weeks 33 to 36 and 37 to 41 total estriol concentrations in amniotic fluid below the level of 200 ng per ml and below the level of 400 ng per ml respectively are indicative of fetal hypotrophy. In the contrary, total estriol concentrations above 500 ng per ml and 1000 ng per ml at weeks 33 to 36 and weeks 37 to 41 respectively are generally not consistent with fetal growth retardation** (see Tab. IV).

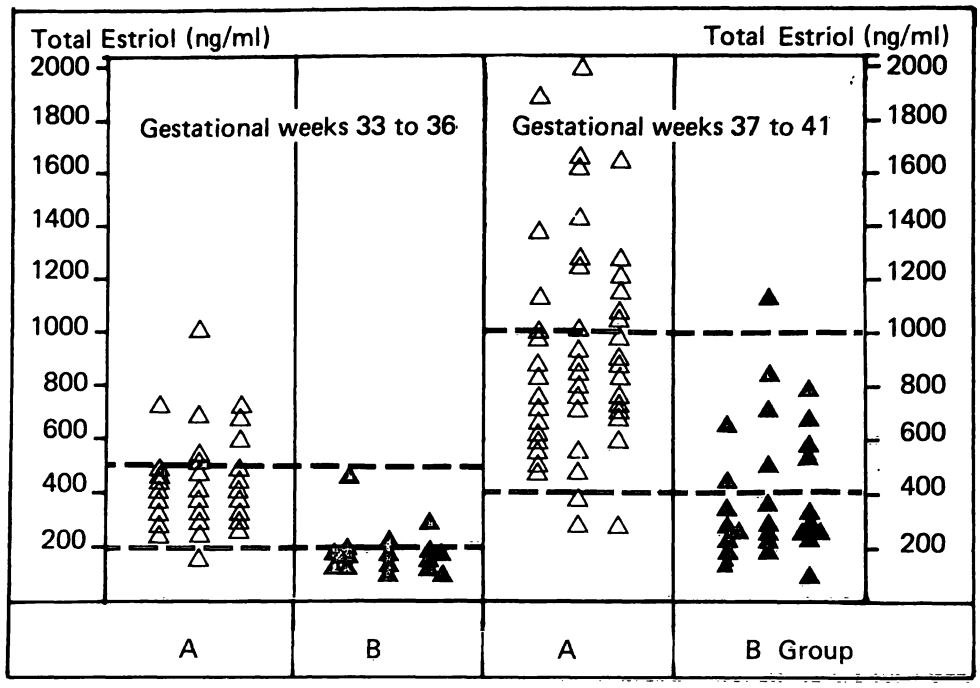

Fig. 4. Distribution within the diagnostic borderlines of total estriol concentrations in amniotic fluid.
Values below the lower borderlines = intrauterine hypotrophy of the infant much likely. Values above the upper borderlines: intrauterine hypotrophy of the infant rather unlikely. A = eutrophic infants, B = hypotrophic infants.

	n	Total estriol (ng/ml) $\bar{x} \pm s$	$\frac{TE\ 3\ \bar{x}\ (B)}{TE\ 3\ \bar{x}\ (A)}$	Mean body weight (g)	$\frac{BW\ \bar{x}\ (B)}{BW\ \bar{x}\ (A)}$	Mean placental weight (g)	$\frac{PW\ \bar{x}\ (B)}{PW\ \bar{x}\ (A)}$
Eutrophic neonates (A)	38	995.8 ± 485.0	—	3274	—	578	—
Hypotrophic neonates (B)	18	466.9 ± 281.5	0.47	2384	0.73	435	0.75

Tab. IV. Total estriol concentrations in amniotic fluid at the day of delivery (TE 3), body weights (BW) and placental weights (PW) in eutrophic and hypotrophic, term babies.

In normal pregnancies numerous investigators have studied estriol levels in amniotic fluid. There are, however, relatively few detailed and representative studies of estriol in amniotic fluid as to special disturbances or diseases in pregnancy [8, 10, 15, 21]. Obviously, estriol assays in amniotic fluid might contribute to antenatal identification of the high-risk hypotrophic fetus, as it could be shown in this study.

Further investigations should be made to clarify which relative predictive value for the antenatal diagnosis of fetal hypotrophy is to be ascribed to estriol assays in amniotic fluid as compared to those in maternal plasma. Comparative determinations of absolute estriol amounts in amniotic fluid in pregnancies with eutrophic and hypotrophic infants might also be interesting. Of course, for such a study simultaneous determinations of the actual estriol concentration and of the actual total volume of amniotic fluid would have to be carried out.

Summary

In 108 pregnancies (gestational weeks 31 to 41) 127 samples of amniotic fluid were assayed for their concentrations of total estriol. Among the pregnancies examined there were 74 pregnancies with eutrophic infants (Group A) and 34 with hypotrophic infants (Group B). The infants were classified in one of the two groups by gestational age and birthweight. Hypotrophic infants were defined as infants whose birthweight lay below the 3rd percentile in the intrauterine growth curves according to NICKL.

Ante partum the samples of amniotic fluid were obtained by transabdominal amniocentesis, sub partu by transcervical amniocentesis. For total estriol assay in amniotic fluid a chemical method was applied which afforded direct quantification of the chromatographically pure estriol spots by means of reflectance spectrophotometry after TLC separation of the estrogenic extracts.

Taken as a whole, total estriol concentrations in amniotic fluid were much lower in pregnancies with hypotrophic infants than in those with eutrophic infants : at gestational weeks 37 to 41 Group A 943.8 ± 464.8 ng per ml, Group B 392.2 ± 258.9 ng per ml, at gestational weeks 33 to 36 Group A 430.0 ± 191.7 ng per ml, Group B 185.7 ± 86.2 ng per ml. The group differences were statistically significant (χ^2-test, $P < 0.001$). As there was a considerable overlapping of the values in both groups, a low normal total estriol concentration in amniotic fluid did not exclude fetal hypotrophy with certainity. Determinate marginal estriol concentrations proved to be of diagnostic and prognostic importance. **At gestational weeks 33 to 36 and 37 to 41 total estriol levels in amniotic fluid below 200 ng per ml and below 400 ng per ml respectively indicated fetal hypotrophy to be much likely. In contrast, values above 500 ng per ml and above 1000 ng per ml at gestational weeks 33 to 36 and 37 to 41 respectively were generally not consistent with hypotrophy of the infants.**

The comparison of the birthweights and total estriol concentrations in amniotic fluid obtained subpartally from eutrophic and hypotrophic term neonates was interesting. The mean relative weight deficit in the hypotrophic newborn infants (27 per cent) was much smaller than their mean relative estriol deficit in amniotic fluid (53 per cent).

The perinatal high risk in the hypotrophic group examined was revealed in the following clinical data : perinatal mortality 8.8 per cent (vs 1.4 per cent in the eutrophic group), respiratory and metabolic acidosis immediately after delivery 29.5 per cent (vs. 12.1 per cent), and depressions of the infants immediately after delivery 32,4 per cent (vs. 12.1 per cent).

In the hypotrophic group, the rate of maternal complications in pregnancy amounted to 55.9 per cent, in the eutropic group, however, only to 5.4 per cent.

Keywords: Amniotic fluid, fetus, fetal hypotrophy, newborn infant, total estriol assays.

Zusammenfassung

Die Rolle von Gesamtöstriol-Bestimmungen im Fruchtwasser zur Diagnose der fetalen Hypotrophie

127 Fruchtwasserproben von 108 Schwangerschaften (Gestationswochen 31–41) wurden auf ihre Konzentrationen an Gesamtöstriol untersucht. Von den untersuchten Schwangerschaften waren 74 Schwangerschaften mit eutrophen Kindern (Gruppe A) und 34 Schwangerschaften mit hypotrophen Kindern (Gruppe B). Die Klassifizierung in eutrophe oder hypotrophe Gruppe richtete sich nach Gestationsalter und Geburtsgewicht. Als hypotrophe Kinder wurden Neugeborene definiert, deren Geburtsgewicht unter der 3. Perzentile der intrauterinen Wachstumskurve nach NICKL lag.

Die Fruchtwasserproben wurden ante partum durch transabdominale Amniozentese gewonnen, sub partu durch Blasensprengung. Zur Bestimmung des Gesamtöstriols im Fruchtwasser wurde eine chemische Methode angewendet, die nach dünnschichtchromatographischer Trennung eine direkte Quantifizierung der chromatographisch reinen Östrolflecke mittels Remissions-Spektrophotometrie gestattet.

Im ganzen gesehen, lagen die Gesamtöstriolwerte im Fruchtwasser bei Schwangerschaften mit hypotrophen Kindern viel niedriger als bei Schwangerschaften mit eutrophen Kindern: in den Schwangerschaftswochen 37–41 Gruppe A 943,8 ± 464,8 ng/ml, Gruppe B 392,2 ± 258,9 ng/ml, in den Schwangerschaftswochen 33–36 Gruppe A 430,0 ± 191,7 ng/ml, Gruppe B 185,7 ± 86,2 ng/ml. Die Gruppendifferenzen waren statistisch signifikant (X^2-Test; $p < 0{,}001$). Da eine beträchtliche Überlappung der Werte beider Gruppen bestand, schloß ein normaler Gesamtöstriolwert im Fruchtwasser eine Hypotrophie des Feten nicht mit Sicherheit aus.

Bestimmte Östriolwerte erwiesen sich als von erheblichem Diagnose- und Prognosewert. **In den Schwangerschaftswochen 33–36 bzw. 37–41 deuteten Gesamtöstriol-Konzentrationen im Fruchtwasser unter 200 ng/ml bzw. 400 ng/ml mit großer Wahrscheinlichkeit auf eine fetale Hypotrophie hin. Demgegenüber schlossen Werte über 500 ng/ml bzw. 1000 ng/ml in den Schwangerschaftswochen 33–36 bzw. 37–41 eine fetale Hypotrophie weitgehend aus.**

Interessant war der Vergleich der subpartualen Gesamtöstriol-Konzentrationen im Fruchtwasser und der Geburtsgewichte von eutrophen und hypotrophen, am Termin geborenen Kindern. Das mittlere relative Gewichtsdefizit (27%) der hypotrophen Neugeborenen war viel geringer als ihr mittleres relatives Östrioldefizit im Fruchtwasser (53%).

Das hohe perinatale Risiko der untersuchten hypotrophen Kinder kam in den folgenden klinischen Daten zum Ausdruck: perinatale Mortalität 8,8% (vs. eutrophe Kinder 1,4%), Depression unmittelbar post partum 32,4% (vs. 5,4%), respiratorische und metabolische Azidosen unmittelbar post partum 29,5% (vs. 12,1%). In der hypotrophen Gruppe betrug die Rate der mütterlichen Schwangerschaftskomplikationen 55,9%, in der eutrophen Gruppe dagegen nur 5,4%.

Schlüsselwörter: Fet, Fruchtwasser, Gesamtöstriol-Bestimmungen, fetale Hypotrophie, Neugeborenes.

Résumé

Le rôle des dosages d'oestriol total du liquide amniotique pour le diagnostic de l'hypotrophie foetale
Le présent article porte sur l'examen des concentrations d'oestriol total de 127 échantillons de liquide amniotique de 108 grossesses (semaine de gestation entre 31 et 41). Sur ces 108 grossesses, 74 mirent à terme des enfants eutrophes (Groupe A) et 34 des enfants hypotrophes (Groupe B). Le classement en groupe eutrophe ou hypotrophe s'opéra d'après l'âge de gestation et le poids à la naissance. On a défini comme enfants hypotrophes les nouveaux-nés dont le poids de naissance était inférieur au 3 pourcentile de la courbe de croissance intrautérine d'après NICKL.
Les échantillons de liquide amniotique ont été obtenus ante partum par amniocentèse transabdominale, sub partu par amniocentèse transcervicale. Pour le dosage d'oestriol total du liquide amniotique, on a utilisé une méthode chimique qui, après séparation de couches minces chromatographique, permet une quantification directe de la tache d'oestriol chromatographiquement pure par spectrophotométrie de rémission.
Considérées dans leur ensemble, les valeurs d'oestriol total du liquide amniotique ont été nettement plus basses dans les grossesses avec enfants hypotrophes que dans celles avec enfants eutrophes: dans les semaines de grossesse 37–41 Groupe A 943,8 ± 464,8 ng/ml, Groupe B 392,2 ± 258,9 ng/ml, dans les semaines de grossesse 33–36 Groupe A 430,0 ± 191,7 ng/ml, Groupe B 185,7 ± 86,2 ng/ml. Les différences entre les groupes ont été statistiquement significatives (X^2-Test; $p < 0{,}001$). Un recoupement très important ayant été observé entre les valeurs des deux groupes, une valeur normale d'oestriol total du liquide amniotique n'exclut pas absolument une hypotrophie du foetus.
Certaines valeurs d'oestriol se sont avérées comme étant d'une grande signification pour le diagnostic et le pronostic. **Dans les 33–36 ou 37–41èmes semaines de grossesse, les concentrations d'oestriol total du liquide amniotique inférieures à respectivement 200 ng/ml ou 400 ng/ml ont indiqué avec un degré de probabilité élevé une hypotrophie foetale. Au contraire, les valeurs supérieures à respectivement 500 ng/ml ou 1000 ng/ml dans les semaines de grossesse entre 33 et 36 ou 37 et 41 ont largement confirmé l'exclusion d'une hypotrophie foetale.**
Une observation intéressante a résulté de la comparaison des concentrations subpartuales d'oestriol total du liquide amniotique et des poids de naissance des enfants eutrophes et hypotrophes nés à terme. Le déficit de poids relatif moyen (27%) des nouveaux-nés hypotrophes s'est montré de beaucoup inférieur à son déficit d'oestriol relatif moyen dans le liquide amniotique (53%).
Le risque périnatal élevé des enfants hypotrophes examinés se manifesta dans les données cliniques suivantes: mortalité périnatale 8,8% (vs' enfants eutrophes 1,4%), dépression directement post partum 32,4% (vs. 5,4%) acidoses respiratoires et métaboliques directement post partum 29,5% (vs. 12,1%). Dans le groupe hypotrophe, le taux des complications de grossesse maternelles s'éleva à 55,9% contre seulement 5,4% dans le groupe eutrophe.

Mots-clés: Dosages d'oestriol total, foetus, hypotrophie foetale, liquide amniotique, nouveau-né.

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